Some Properties of a Cell Line and its Clones Established from the Hepatocellular Carcinoma Induced in the Rat by 2-Acetylaminofluorene

— Effect of Dexamethasone —

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SUMMARY

We have established the cell line designated as FAA-HTCl and the two clones from the well-differentiated hepatocellular carcinoma which was induced in a male F344/DuCrj rat fed 0.02% 2-acetylaminofluorene for 10 weeks followed by feeding a basal diet for 42 weeks. The cells of the parent line were maintained in a culture medium consisting of Williams' medium E and 10% fetal bovine serum, and its clones were maintained in the culture medium supplemented with 10^{-6} M dexamethasone (Dex). These parent and clonogenic cells were unique with respect to the fact that viability was decreased in exponential growth phase followed by remaining in a low viability in plateau phase, and failure to reach the confluence. The treatment for not exceeding 21 days with 10^{-6} M Dex did not affect these growth properties. However, the Dex treatment for 4 weeks reduced the seeding and plating efficiency and lengthened the population doubling time. The clone 1 cells were similar in these growth indices to the Dex-treated parent cells, while the clone 2 cells displayed the indices similar to the untreated parent cells, suggesting clonogenic variations. A small population of the parent cells had cytochemical activities of glucose 6-phosphatase, alkaline phosphatase and γ-glutamyl transpeptidase (GGT). The parent cells also had biochemical activities of tyrosine aminotransferase, GGT and catalase. Except for catalase, a marked enhancement

* Abbreviations:

HTC,	hepatocellular carcinoma;	G-6-Pase,	glucose 6-phosphatase;
Dex,	dexamethasone;	WME,	Williams' medium E;
HIFBS,	heat-inactivated fetal bovine serum;	HBSS,	Hanks' balanced salt solution;
AFP,	α-fetoprotein;	Al-Pase,	alkaline phosphatase;
GGT,	γ-glutamyl transpeptidase;	TAT,	tyrosine aminotransferase.

of activities of the above mentioned enzymes were detected in the parent cells treated with Dex for 4 weeks. Peroxisomes of the parent cells were clustered in their cytoplasms, while in the greater majority of the Dex-treated cells, peroxisomes were evenly distributed. Immunocytochemically, the parent cells as well as the Dex-treated parent cells were found to have albumin, α-fetoprotein and the cytokeratins (PKK1). In addition, the parent cells had vimentin, while the greater majority of the Dex-treated cells did not have vimentin. Although clonogenic variations were detected, the cells of clones 1 and 2 showed the cytochemical and biochemical activities of the above mentioned enzymes at a level similar to that of the Dex-treated parent cells, except for catalase activity. In the cells of clones 1 and 2, catalase activity was higher than that of the parent cells and peroxisomes were clustered. The immunocytochemical properties of these clonal cells were similar to those of the Dex-treated parent cells.

Key words: Hepatocellular carcinomas, Cell cultures, Dexamethasone.

INTRODUCTION

A number of studies have been concerned with the improvements of primary culture system of adult hepatocytes for the long-term maintenance of hepatocytic functions or for enhancing proliferation capability of the cells. However, we have not succeeded as yet to maintain for more than one month the cultured cells with the functions at a level comparable to that of the cells *in vivo,* or to acquire continuous proliferation of the cells. As an alternative system to investigate hepatic functions in culture, established lines of well-differentiated hepatocellular carcinoma (HTC*) cells may be useful, because that they could proliferate continuously and express hepatocytic functions.

We have previously established the cell lines from Morris hepatoma 7316A, a transplantable HTC of the rat(10). These lines were used for studying the effects of dexamethasone (Dex) and insulin on induction and suppression of glucose 6-phosphatase (G-6-Pase) activity(25) and on the changes in peroxisomal distribution and induction of crystalloid nucleoids in peroxisomes(10, 11). We present here the establishment of the cell line and its clones from the well-differentiated HTC which was induced in the rat fed the genotoxic carcinogen, 2-acetylaminofluorene. Moreover, some properties of the cells, including those related to hepatocytic functions, and the effect of Dex on these phenotypes are investigated. The induction by Dex of the hepatocytic functions in culture was documented well in the previous studies(1, 2, 4, 6, 8, 11, 13, 14, 17, 23, 25).

MATERIALS AND METHODS

Establishment of a Rat Hepatocellular Carcinoma Cell Line and its Clones.

The HTC cell line designated as FAA-HTC1 was obtained from the liver tumor of a male F344/DuCrj rat which was fed 0.02% 2-acetylaminofluorene in the basal diet (Oriental Yeast, Tokyo) for 10 weeks followed by feeding the basal diet for 42 weeks. The liver was perfused with collagenase and dispase II as described previously(12), followed by excision of tumor portions. The solutions used for the perfusion, cell dissociation, centrifugation, culturing and subculturing contained 40 U/m*l* penicillin G and 40 μg/m*l* streptomycin sulfate. A part of the excised tumor was processed for histological examinations and the rest was minced with fine scissors. The cells were dissociated from the minced tumor fragments by the incubation for 30 min at 37℃ with 600 PU/m*l* dispase II in Williams' medium E (WME) (Flow Labs., Irving, UK) containing 10 mM HEPES using a rotary shaker. The suspension containing dissociated cells and undissociated tumor tissue fragments was filtered through a double-layer of gauzes and subsequently through a nylon mesh of 250 μm pore size, followed by centrifugation at 50×g for 1 min. The sedimented cells were then washed three times with WME supplemented with 10% heat-inactivated calf serum (GIBCO, Grand Island, USA). The cells were finally suspended in WME supplemented with 10% heat-inactivated fetal bovine serum (HIFBS) (Hyclone Labs., Logan, USA) and 10^{-6} M Dex (Sigma) (WME: $HIFBS_{10}$: Dex) and seeded in 60×15 mm tissue culture dishes (Corning; Iwaki Glass, Tokyo). The cells in the primary culture were maintained for 14 days in WME: $HIFBS_{10}$: Dex. A mixture of colonies of HTC cells and fibroblast-like cells were obtained in a few dishes. The cells in one dish were subcultured to the first passage (P 1) culture using Ca^{++} and Mg^{++}-free Hanks' balanced salt solution (HBSS) containing 0.005% EDTA-Na_2 as the washing solution and 0.01% trypsin (2914 NF units/mg; GIBCO) in Ca^{++} and Mg^{++}-free HBSS containing 0.01% EDTA-Na_2 for the enzymatic dissociation. The culture medium for P 1 and subsequent passage cultures was WME containing 10% HIFBS without Dex (WME: $HIFBS_{10}$), and the trypsinization was used for further subculturing.

It was observed in the P 7 to P 9 cultures in the plateau phase that a great number of cells were floating. Although viable cells were not detected in the media by the trypan blue dye exclusion method, the medium containing floating cells in a 60-mm dish at Day 14 of P 9 culture was diluted 10 times with the fresh medium and transferred to 10 of 60-mm dishes. At Day 7, 1 to 3 colonies of HTC cells without fibroblast-like cells were observed in 8 of 10 dishes, and the dish containing one colony was selected and maintained. The cells in this dish were subcultured at Day 14 to P 10 cultures at 1.0×10^4 cells/60-mm dish, or at 5 cells/35-mm dish for

cloning. Two clones were obtained by this dilution method, and the medium was changed to WME : HIFBS10 : Dex at Day 1, followed by maintaining the clonal cells in this culture medium. Thus, the parent FAA-HTC1 cell line and its clones 1 and 2 were established. Parent FAA-HTC1 cells were maintained in WME : $HIFBS_{10}$. The parent and clonal cells were fed the fresh media twice a week. The seeding density for maintaining cultures was 1.0×10^4 cells/60-mm dish or 1.0×10^5 cells per T-75 flask. Subculturing by trypsinization was carried out every 2 weeks. Properties of parent FAA-HTC1 cell line and its clones were investigated using the cells in the P 11 to P 19 cultures. Effects of Dex on parent FAA-HTC1 cells were examined by maintaining the cells in WME : $HIFBS_{10}$: Dex. There were no mycoplasmal infections, that was examined with Hoechst Stain Kit (Flow).

Chemicals and Antibodies.

All chemicals and antibodies were purchased from the commercial sources as follows: α-ketoglutarate (monopotassium salt), pyridoxal phosphate (monohydrate) and L-tyrosine from Wako Pure Chemicals Indust. (Tokyo); 3, 3'-diaminobenzidine from Tokyo Kasei (Tokyo); γ-L-glutamyl-*p*-nitroanilide and glycylglycine from Katayama Chemicals (Osaka); fast blue BBN salt, fast blue RR salt, glucose 6-phosphate, naphthol AS-MX phosphate-Na and L-homoarginine from Sigma Chemical Co. (St. Louis, USA); γ-L-glutamyl-4-methoxy-β-naphthylamide from VEGA Biochomicals (Tucson, USA); anti-rat albumin IgG fraction (sheep) from Cappel Labs. (West Chester, USA); anti-rat α-fetoprotein (AFP) IgG fraction (sheep) from Nordic Immunological Labs. (Tilburg, Netherlands); mouse monoclonal IgG to cytokeratins-PKK1 from Labsystems Oy (Helsinki, Finland); mouse monoclonal IgG to vimentin from DAKOPATTS a/s (Glostrup, Denmark); Vectastain ABC sheep and mouse IgG Kits and Blocking Kits from Vector Labs. (Burlingame, USA).

Cytochemical and Immunocytochemical examinations.

The activity of G-6-Pase was examined cytochemically by the method of Wachstein and Meisel(27). Alkaline phosphatase (Al-Pase) activity was examined by the azo-dye method of Burstone(5). γ-Glutamyl transpeptidase (GGT) activity was determined according to the method of Rutenburg *et al.*(24). Peroxisomes were examined light and electron microscopically by the method of Novikoff *et al.*(19) for detection of catalase activity in peroxisomes. Immunocytochemistries of albumin, AFP and the intermediate filament proteins, cytokeratins-PKK1 and vimentin, were carried out by the immunoperoxidase staining method using avidin-biotin-peroxidase complex (Vectastain ABC Kits).

Biochemical assays of enzyme activities.

All steps to prepare cell homogenates were carried out at 0 to 4℃. The cells were harvested with a rubber policeman and suspended in distilled water followed by homogenization with 15 strokes of a Potter-Elvehjem homogenizer. Tyrosine aminotransferase (TAT) activity was measured by the method of Diamondstone (7). A unit of the enzyme activity was defined as conversion of 1 nmol of *p*-hydroxyphenylpyruvic acid to *p*-hydroxybenzaldehyde per min at 25℃. GGT activity was determined by the method of Novogrodsky *et al.*(20). One unit of the activity was defined as the amount of GGT catalyzing formation of 1 μmol *p*-nitroaniline per min at 25℃. Catalase activity was measured according to the spectrophotometric method of Beers and Sizer(3). A unit of catalase was defined as the amount of catalase decomposing 1 μmol of hydrogen peroxide per min at 25℃. Protein contents of cell homogenates were determined by the method of Lowry *et al.* (16) using bovine serum albumin as the standard.

RESULTS

Growth properties.

Growth curve and changes in viability of the parent FAA-HTC1 cells and the effect of the treatment with Dex on them are illustrated in Fig. 1. At Day 7 of the culture, when the cells were in exponential growth phase, a certain number of non-viable cells were detected, followed by an increase in the number of non-viable cells as culture aged. At Days 11 to 21, the viable cells were in a plateau phase. Non-viable cells were in a plateau phase at Days 17 to 21. Even in the plateau phase, the cells never reached the confluence, and a large number of floating non-viable cells were observed in the aged cultures. The saturation density of the viable cells were approximately 4.5×10^5 to 9.5×10^5 cells/60-mm dish, while the density of non-viable cells were 4.5×10^5 to 7.0×10^5 cells/dish. Viability of the cells in the plateau phase was constantly in a range of 40 to 60%. The short-term treatment with Dex did not affect the growth and changes in viability of the parent cells, as shown in Fig. 1. These characteristics were also found in the cultures of clones 1 and 2 (not shown).

Seeding efficiency, plating efficiency and population doubling time of the parent line and its clones are listed in Table 1. The seeding efficiency of the parent cells which had been treated for 4 weeks with 10^{-6} M Dex was lower than that of their untreated counterparts. The clone 1 cells displayed a seeding efficiency lower than that of the untreated parent cells. The efficiency of the clone 2 cells was similar to that of the untreated parent cells and much higher than that of the Dex-treated parent cells and clone 1 cells. The plating efficiency of the parent cells treated for 4 weeks and 11 days in the subculture was lower than that of their untreated

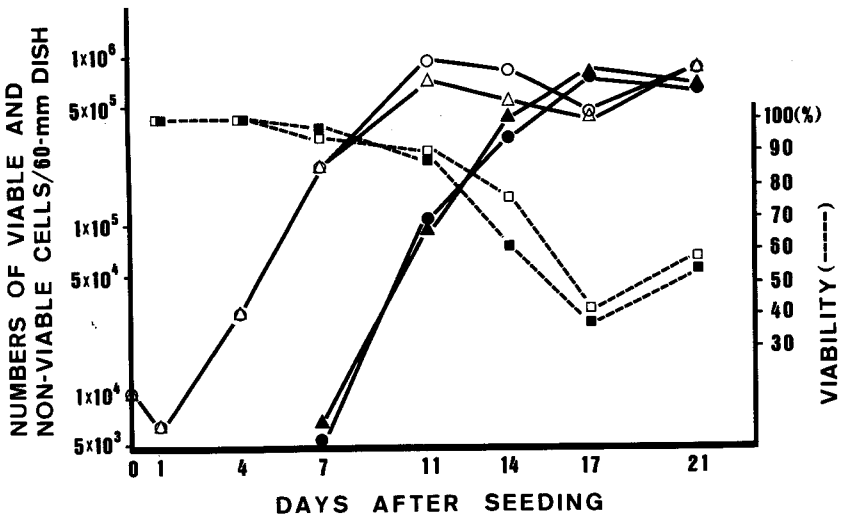

Fig. 1 Growth curve and changes in viability of FAA-HTC1 line (P 11) maintained with or whthout Dex. ○—○, The mean viable cell number/60-mm dishmaintained in WME : $HIFBS_{10}$. △—△, The mean viable cell number/dish when maintained in WME : $HIFBS_{10}$: Dex. The treatment with 10^{-6}M Dex was started at 4 hr of the P 11 culture. ●—●, The means of non-viable cell number/dish maintained in WME : $HIFBS_{10}$; ▲—▲, the means of non-viable cell number/dish when maintained in WME : $HIFBS_{10}$: Dex. □-----□, The mean viability of the cells maintained in WME : $HIFBS_{10}$; ■-----■, the mean viability of the cells when maintained in WME : $HIFBS_{10}$: Dex. The seeding density was 1.0×10^4 viable cells/dish. Values are derived from triplicates, each consisting of 3 dishes.

Table 1 *Growth properties of a rat hepatocellular carcinoma cell line, FAA-HTC1, and its clones.*

	FAA-HTC1 parent			
	Control	10^{-6}M Dex[a]	Clone 1	Clone 2
Seeding efficiency (%)[b]	73±14	50±3*	43±2*	79±12
Plating efficiency (%)[c]	17± 1	13±3**	8±1***	21± 1***
Population doubling time (hr)[d]	27.5	34.5	37.6	22.1

[a] Dex, dexamethasone. Clones 1 and 2 were maintained in the culture medium supplemented with 10^{-6}M Dex.

[b] The seeding density was 2.0×10^5 cells/60-mm dish. The number of viable attached cells per dish was determined at 22 hr by harvesting the cells with trypsin. Values are means±SD and derived from triplicates, each consisting of 2 dishes.

[c] The seeding density was 500 cells/60-mm dish. The number of colonies/dish was determined at Day 11. Values are mans±SD and derived from 10 dishes.

[d] The seeding density was 3.0×10^4 cells/60-mm dish. The number of viable attached cells was determined at Days 5 and 7. Values are means and derived from triplicates at each counting day, each consisting of 3 dishes.

* The significance level as compared with values for control FAA-HTC1 parent cells maintained in WME : $HIFBS_{10}$; $p<0.05$.

** The significance level as compared with values for the control parent cells ; $p<0.005$.

*** The significance level as compared with values for the control parent cells ; $p<0.001$.

counterparts. The cells of clones 1 and 2 were lower and higher, respectively, in the plating efficiency than the untreated parent cells. The population doubling time of the parent cells was delayed by the treatment with Dex for 4 weeks. The clone 1 cells exhibited a doubling time similar to that of the Dex-treated parent cells, while the clone 2 cells had a doubling time slightly shorter than that of the untreated parent cells.

Morphological and cytochemical properties.

The hepatic tumor used for cell isolation was histologically a well-differentiated HTC. As shown in Fig. 2A, some of the HTC cells had glycogen. Morphology of the parent FAA-HTC1 line and its clones are shown in Figs. 2B, 2C and 2D. The parent cells were in a spherical shape, and their cytoplasms did not well spread out, even in the periphery of a colony (Fig. 2B). Whereas, the clone 1 cells exhibited an epithelial appearance with polygonal shape (Fig. 2C). The epithelial morphology similar to the clone 1 cells was observed in the great majority of the clone 2 cells (Fig. 2D), while the colonies formed by the cells having a morphology similar to that

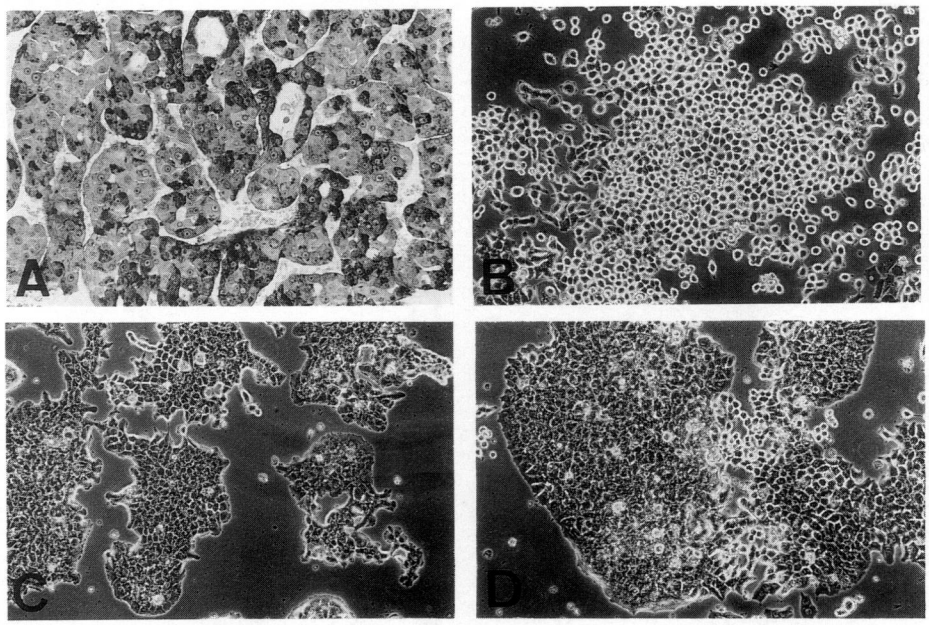

Fig. 2 A, The well-differentiated hepatocellular carcinoma of the rat fed 2-acetylaminofluorene, that was used for the cell isolation. PAS staining, ×100. The tumor cells containing PAS-positive glycogen particles are seen. B-D, Phase contrast microphotographs of the cultured HTC cells, ×50. B, The parent FAA-HTC1 cells; C, the clone 1 cells maintained in WME : $HIFBS_{10}$: Dex; D, the clone 2 cells maintained in the Dex-supplemented medium.

of the parent cells were also found in the clone 2 cell cultures. The parent cells maintained for 4 weeks in the Dex-supplemented medium had morphologic characteristics similar to those of the clone 2 cells.

To investigate hepatocytic properties of the cultured HTC cells, the cytochemistries of G-6-Pase, Al-Pase and GGT (Figs. 3 and 4) were carried out. Low frequency of the cells positive in these enzyme activities was detected in the parent cells. (Figs. 3A, 3E and 4A). In cultures of the parent cells treated for 4 weeks with 10^{-6} M Dex, frequency of the cells positive in the cytochemical activities was

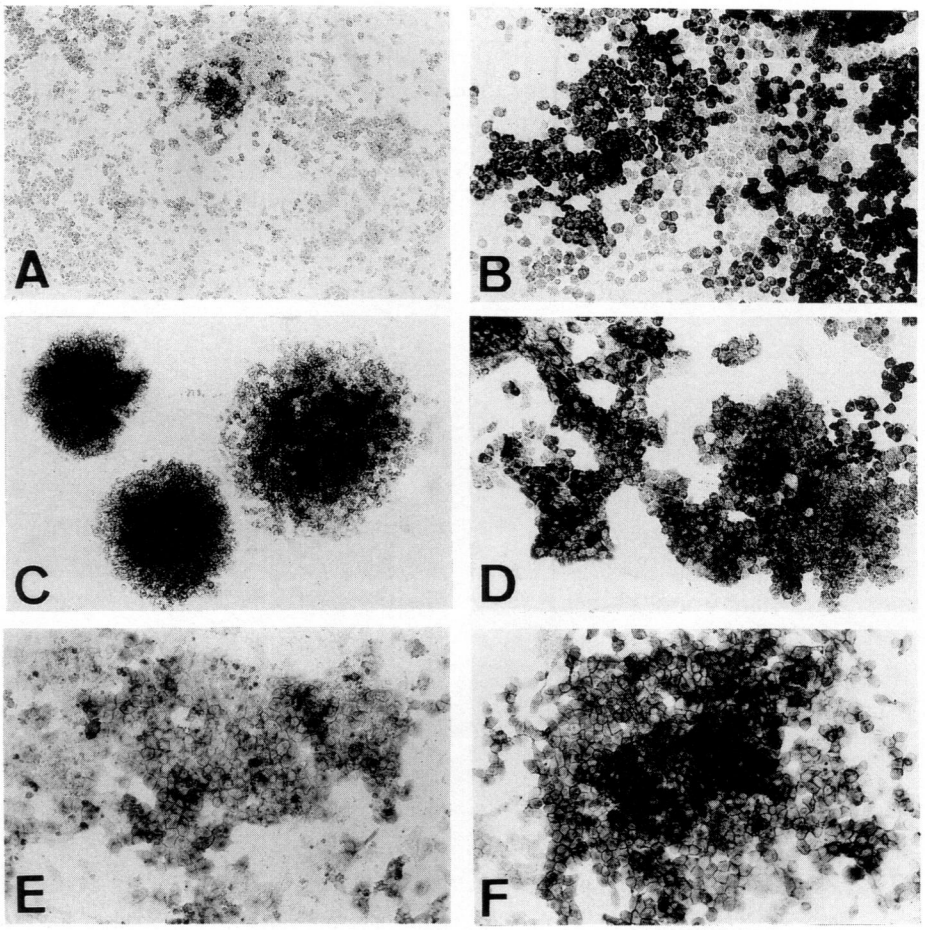

Fig. 3 Cytochemical G-6-Pase and Al-Pase activities of parent FAA-HTC1 line and its clones. A-D, G-6-Pase activity; E and F, Al-Pase. A and E, The parent cells, ×25.6 and ×64, respectively. B and F, The parent cells maintained for 4 weeks in WME : $HIFBS_{10}$: Dex, ×64. C, The clone 1 cells, ×64; D, the clone 2 cells, ×64.

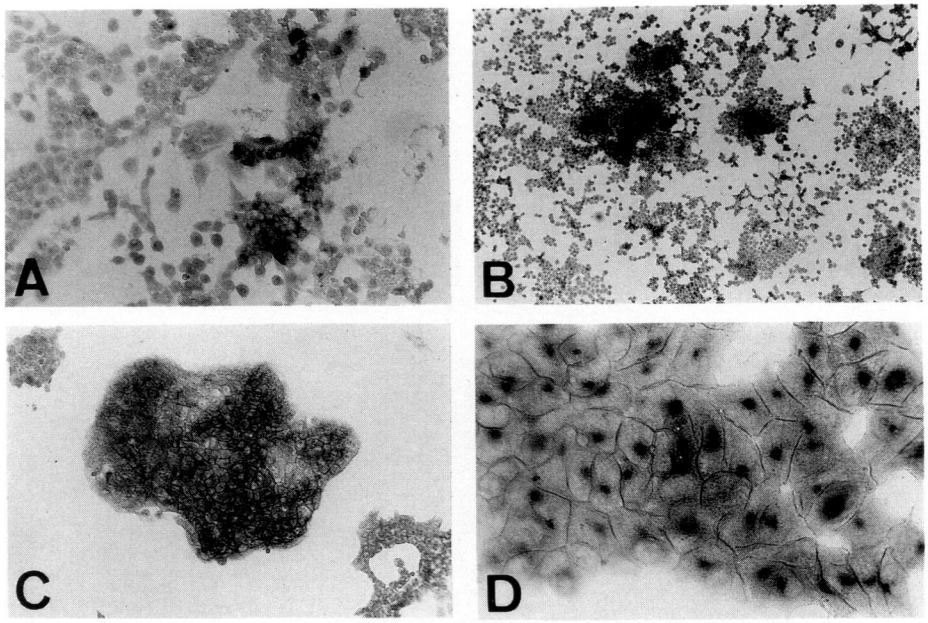

Fig. 4 Cytochemical GGT activity of parent FAA-HTC1 line and its clones. A, The parent cells, ×64; B, the parent cells maintained for 4 weeks in WME : $HIFBS_{10}$: Dex, ×25.6; C, the clone 1 cells, ×64; D, the clone 2 cells, ×160. Focal areas on the cell membranes exhibiting high GGT activity are seen in D.

much greater than that of the untreated counterparts, and in case of Al-Pase, the activity of the positive cells was also much higher than that of the untreated counterparts (Figs. 3B, 3F and 4B). The cells of clones 1 and 2 exhibited the high cytochemical activities of G-6-Pase and GGT (Figs. 3C, 3D, 4C and 4D). As shown in Fig. 4D, some cell populations of the Dex-treated parent cells and the cells of clones 1 and 2 showed focal areas of markedly high activity of GGT on their membranes. The Al-Pase activity of the cells of the two clones was in a level similar to that of the Dex-treated parent cells (not shown).

Peroxisomes of the parent cells were densely clustered in the cytoplasms and formed a large condensed body, as shown in Fig. 5A. Ultrastructurally, peroxisomes were found to be clustered mainly around lipid droplets (Fig. 5B). Whereas, in the majority of the Dex-treated parent cells, peroxisomes were evenly distributed in the cytoplasms, as shown in Fig. 5C. In addition, peroxisomes located along large vacuoles were detected in the minor populations of the cells (Fig. 5D). Peroxisomes in the cells of the two clones displayed clustering similar to that of the parent untreated cells. Crystalloid nucleoids were not found in peroxisomes of the parent cells.

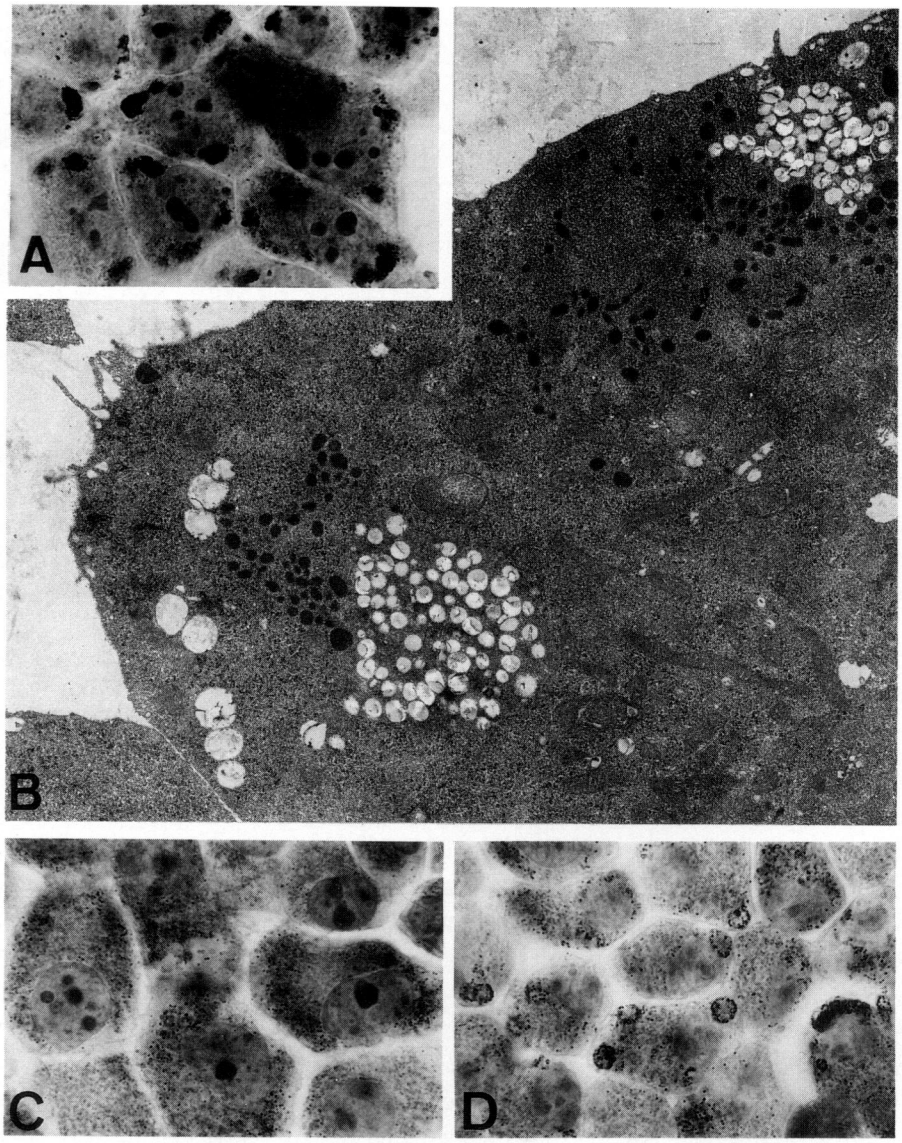

Fig. 5 Light and electron microphotographs of catalase cytochemistry of parent FAA-HTC1 cells. A and B, The cells maintained in WME : $HIFBS_{10}$. A, Oil immersion, ×640. Clusters of the reaction-positive peroxisomes are seen. B, the electron microscopic cytochemistry, ×8,000. Peroxisomes are clustered around focal areas of lipid droplets. C and D, The cells maintained for 4 weeks in WME : $HIFBS_{10}$: Dex, oil immersion, ×640. Peroxisomes in the majority of the cells were evenly distributed in the cytoplasms as seen in C, while peroxisomes in a few cells were located along large vacuoles as seen in D.

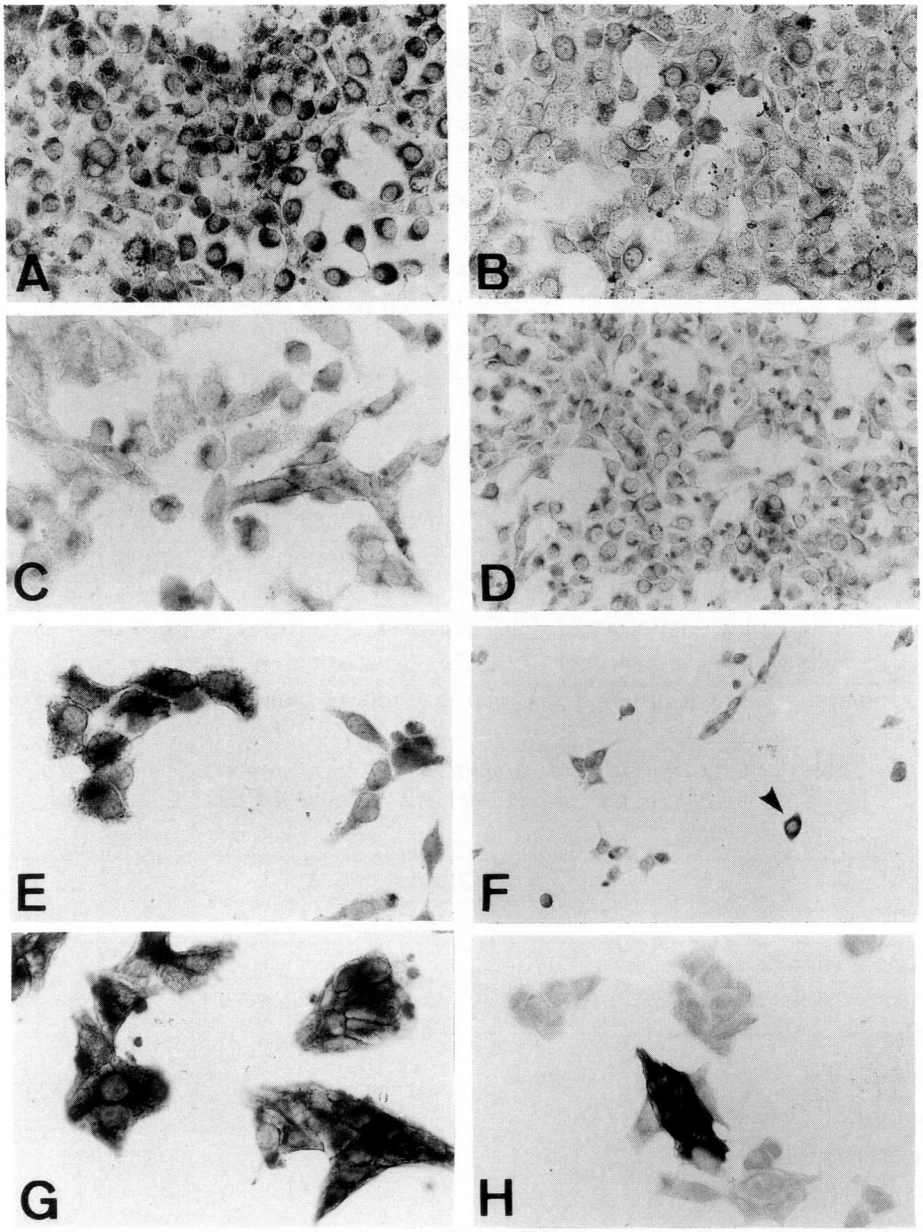

Fig. 6 Immunoperoxidase staining of parent FAA-HTC1 line and its clones using avidin-biotin complex. A and B, The cells are counterstained with methyl green, ×160; C-F, the cells are counterstained with hematoxylin. A-D, The parent cells; E and F, the parent cells maintained for 4 weeks in WME : $HIFBS_{10}$: Dex; G and H, the clone 1 and clone 2 cells, respectively. A, Albumin; B, AFP; C, E and G, cytokeratins-PKK1, ×200; D, F and H, vimentin, ×100, ×100 and ×200, respectively. In F, one cell (an arrow head) is positive in vimentin, while the rest was negative.

Immunocytochemical properties.

As shown in Figs. 6A and 6B, the parent cells produced albumin and AFP. The parent cells treated for 4 weeks with Dex and the clonal cells were also positive in both albumin and AFP. The parent cells had the cytokeratins and vimentin (Figs. 6C and 6D). The treatment for 4 weeks with Dex did not alter the distribution of the cytokerations in the parent cells (Fig. 6E). In contrast, the frequency of the cells positive in vimentin was very low in cultures of the Dex-treated parent cells (Fig. 6F). The cells of clones 1 and 2 were positive in cytokeratins-PKK1 (Fig. 6G), and similar to the case of the Dex-treated parent cells, the frequency of the vimentin-positive cells was low (Fig. 6H).

The cytochemical and immunocytochemical properties of the parent line and its clones are summarized in Table 2.

Bicochemical properties.

Biochemical activities of TAT, GGT and catalase of the parent line and its clones are listed in Table 3. TAT and GGT activities of the parent cells were markedly encanced by the treatment for 4 weeks with Dex. The cells of clones 1 and 2 exhibited high activities of TAT and GGT. However, TAT activity of the clone 1 cells was lower than that of the Dex-treated parent and clone 2 cells, and GGT activity was not different between the cells of clones 1 and 2, but the two

Table 2 *Cytochemical and immunocytochemical properties of a rat hepatocellular carcinoma cell line, FAA-HTC1, and its clones.*

	FAA-HTC1 parent			
	Control	10^{-6}M Dex[a]	Clone 1	Clone 2
Glucose 6-phosphatase	±[b]	+++[c]	+++	+++
Alkaline phosphatase	±	+++	+++	+++
γ-Glutamyl transpeptidase	±	+++	+++	+++
Peroxisomes	Clustered	evenly distributed	clustered	clustered
Albumin	+[d]	+	+	+
α-Fetoprotein	+	+	+	+
Cytoperatins-PKK1	+	+	+	+
Vimentin	+	±	±	±

[a] Dex, dexamethasone. Clones 1 and 2 were maintained in the culture medium supplemented with 10^{-6}M Dex.

[b] A very small population of the cells was positive.

[c] The greater majority of the cells exhibited a strong positivity.

[d] All of the cells were positive.

Table 3 *Some biochemical properties of a rat hepatocellular carcinoma cell line, FAA-HTC1, and its clones.*

	FAA-HTC1 parent			
	Control	10^{-6}M Dex[a]	Clone 1	Clone 2
Tyrosine aminotransferase	10 ±5[b]	91± 4*	55± 5*,†	96± 3*
γ-Glutamyl transpeptidase	4.3±1.0	12± 1*	41±16***,††	33±11***,††
Catalase	62 ±6	65±10	101± 8**	88±10***

[a] Dex, dexamethasone. Clones 1 and 2 were mintained in the culture medium supplemented with 10^{-6}M Dex.

[b] Values are means±SEM of triplicates. Each assay was carried out in duplicate.

* The significance level as compared with values for control FAA-HTC1 parent cells maintained in WME : $HIFBS_{10}$; $p<0.001$.

** The significance level as compared with values for the control parent cells ; $p<0.005$.

*** The significance level as compared with values for the control parent cells; $p<0.025$.

† The significance level as compared with values for the Dex-treated parent cells ; $p<0.001$.

†† The significance level as compared with values for the Dex-treated parent cells ; $p<0.05$.

clonogenic cells were higher in GGT activity than the Dex-treated parent cells. Catalase activity of the parent cells was not altered by the Dex-treatment. The cells of clones 1 and 2 were higher in catalase activity than the parent cells.

DISCUSSION

The cell line established from the well-differentiated hepatocellular carcinoma, FAA-HTC1, and its clones 1 and 2 have a unique growth property. In the exponential growth phase, viability was decreased with culture age, followed by remaining at a low level in the plateau phase. The decrease in viability in the growth phase has not been reported previously. On the other band, the cells did not reach the confluence. This property was also found in the cell line established from Morris cepatoma 7316A(10) and in the cell strains derived from Morris hepatomas 7795 and 7800(21, 22). The short-term treatment with Dex after seeding did not affect these growth properties of the parent cells. However, the Dex-treatment for more than 4 weeks decreased seeding and plating efficiency, and lengthened the population doubling time. The clone 1 cells displayed these growth indices similar to those of the Dex-treated parent cells, while the clone 2 cells were similar in the indices to the untreated parent cells. The suppression by Dex of growth of the parent cells may not always expressed in its clones.

The expression and induction by Dex of the activities of G-6-Pase, Al-Pase, GGT and TAT have been demonstrated in the cultures including hepatocyte primary culture and hepatoma cell culture(1, 2, 4, 6, 8, 13, 14, 17, 23, 25). The parent lines

had small population of the cells positive in the cytochemical activities or exhibited the detectable activities of these enzymes. A remarkable enhancement by Dex of these enzyme activities was clearly demonstrated in the Dex-treated parent cells. Moreover, the cells of clones 1 and 2 also displayed the activities of these enzymes at a level similar to that of the Dex-treated parent cells, although the level in the two clones were not identical. These results suggest that the cells of FAA-HTC1 line and its clones express the hepatocytic functions, although clonogenic variations are detected.

Clustering of anucleoid peroxisomes around lipid droplets in the parent cells suggests a close association between lipid metabolism and peroxisomes, which contain the fatty acyl Co-A oxidizing system different from that of mitochondria (15). Whereas, in the cells treated with Dex, peroxisomes were evenly distributed or located along large vacuoles which might be formed by the fusion of lipid droplets. On the other hand, peroxisomes of the cells of clones 1 and 2 were also clustered in a similar way to that of the parent control cells, suggesting clonogenic variation of peroxisomal responsiveness to Dex. The effect of Dex on the changes in distribution of peroxisomes has also been observed in the Morris hepatoma 7316A cells(11). The cells of clones 1 and 2 were higher in catalase activity than the parent cells, suggesting the enhancing effect of Dex on the activity. In contrast, the activity of the Morris hepatoma cells was suppressed by Dex and enhanced by insulin, and the formation of crystalloid nucleoids was induced by Dex(11). It is expected that the cells of FAA-HTC1 line and its clones regulate peroxisomal metabolism through a pathway different from that of the Morris hepatoma cells. Further studies on peroxisomes in the cultured HTC cells may produce new findings regarding peroxisomal lipid metabolism or the synthesis and the degradation mechanisms of the organelles.

The immunoperoxidase stainings demonstrated that the parent cells and the cells of the two clones produced the hepatocytic proteins, albumin and AFP. Dex did not affect the production of these serum proteins in the parent and the clonal cells. Cytokeratins are the intermediate filament proteins of epithelial cells and carcinomas(18). They were found in the non-transformed liver epithelial cells, hepatoma cells and mouse hepatocytes in the primary culture(9, 18, 26). We observed that adult rat hepatocytes in primary culture were positive in the cytokeratins-PKK1 and vimentin (unpublished observations). Interestingly, vimentin-positive cells were found in a low frequency in cultures of the Dex-treated parent cells, clones 1 and 2 cells. This suppression might be associated with the emergence of the clonogenic cells with a epithelial morphology in the Dex-treated cell cultures.

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REFERENCES

1. ALEXANDER, R. W. and GRISHAM, J. W.: Lab. Invest. **22**, 50-62 (1970).
2. AUERBACH, V. H. and WALKER, D. L.: Biochim. Biophys. Acta **31**, 268-269 (1959).
3. BEERS, R. F. Jr. and SIZER, I. W.: J. Biol. Chem. **195**, 133-140 (1952).
4. BOXER, J., KIRBY, L. T. and HAHN, P.: Proc. Soc. Exp. Biol. Med. **145**, 901-903 (1974).
5. BURSTONE, M. S.: J. Histochem. Cytochem. **9**, 146-153 (1961).
6. DIAMOND, L., MCFALL, R., TASHIRO, Y. and SABATINI, D.: Cancer Res. **33**, 2627-2636 (1973).
7. DIAMONDSTONE, T. I.: Anal. Biochem. **16**, 395-401 (1966).
8. EDWARDS, A. M.: Cancer Res. **42**, 1107-1115 (1982).
9. FRANKE, W. W., DENK, H., KALT, R. and SCHMID, E.: Exp. Cell Res. **131**, 299-318 (1981).
10. FURUKAWA, K., MOCHIZUKI, Y., GOTOH, M., SAWADA, N. and TSUKADA, H.: Tumor Res. **15**, 24-34 (1980).
11. FURUKAWA, K., MOCHIZUKI, Y. and TSUKADA, K.: Proc. XIth Int. Cong. on Electron Microscopy, 2585-2586 (1986).
12. FURUKAWA, K., SHIMADA, T., ENGLAND, P., MOCHIZUKI, Y. and WILLIAMS, G. M.: In Vitro Cell. Develop. Biol. **23**, 339-348 (1987).
13. GERSCHENSON, L. E., ANDERSSON, M., MOLSON, J. and DAVIDSON, M. B.: Science **170**, 859-861 (1970).
14. JUNG, W., GEBHARDT, R. and MECKE, D.: Eur. J. Cell Biol. **27**, 230-241 (1982).
15. LAZAROW, P. B. and De DUVE, C. A.: Proc. Natl. Acad. Sci. USA **73**, 2043-2046 (1979).
16. LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L. and RANDALL, R. J.: J. Biol. Chem. **193**, 265-275 (1951).
17. MICHALOPOULOS, G., SATTLER, G. L. and PITOT, H. C.: Cancer Res. **38**, 1550-1555 (1978).
18. MOLL, R., FRANKE, W. W., SCHILLER, D. L., GEIGER, B. and KREPLER, R.: Cell **31**, 11-24 (1982).
19. NOVIKOFF, A. B., NOVIKOFF, P. M., DAVIS, C. and QUINTANA, N.: J. Histochem. Cytochem. **21**, 737-755 (1973).
20. NOVOGRODSKY, A., TATE, S. S. and MEISTER, A.: Proc. Natl. Acad. Sci. USA **73**, 2414-2419 (1976).
21. RICHARDSON, U. I., TASHJIAN, A. H. Jr. and LEVINE, L.: J. Cell Biol. **40**, 236-247 (1969).
22. RICHARDSON, U. I., SNODGRASS, P. J., NUZUM, C. T. and TASHJIAN, A. H. Jr.: J. Cell. Physiol. **83**, 141-150 (1974).

23. ROSENBERG, M. R., STROM, S. C. and MICHALOPOULOS, G.: In Vitro **18**, 775-782 (1982).
24. RUTENBURG, A. M., KLM, H., FISCHBEIN, J. W., HANKER, J. S., WASSERKRUG, H. L. and SELIGMAN, A. M.: J. Histochem. Cytochem. **17**, 517-526 (1969).
25. SAWADA, N., FURUKAWA, K., MOCHIZUKI, Y., GOTOH, M. and TSUKADA, H.: Tumor Res. **14**, 1-8 (1979).
26. SWIERENGA, S. H., GOYETTE, R. and MARCEAU, N.: Exp. Cell Res. **153**, 39-49 (1984).
27. WACHSTEIN, M. and MEISEL, E.: J. Histochem. Cytochem. **4**, 529 (1956).